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Glucose and Lipid Metabolism—Review

Changes in Lipoprotein Lipase in the Heart Following Diabetes Onset

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ABSTRACT

Due to its constant pumping and contraction, the heart requires a substantial amount of energy, with fatty acids (FAs) providing a major part of its adenosine triphosphate (ATP). However, the heart is incapable of making this substrate and attains its FAs from multiple sources, including the action of lipoprotein lipase (LPL). LPL is produced in cardiomyocytes and subsequently secreted to its heparan sulfate proteoglycan (HSPG) binding sites on the plasma membrane. To then move LPL to the endothelial cell (EC) lumen, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) attaches to interstitial LPL and transfers it to the vascular lumen, where the LPL is ready to perform its function of breaking down circulating triglycerides (TG) into FAs. The endo-β-glucuronidase heparanase (Hpa) is unique in that it is the only known mammalian enzyme to cleave heparan sulfate (HS), thereby promoting the abovementioned release of LPL from the cardiomyocyte HSPG. In diabetes, it has been suggested that changes in how the heart generates energy are responsible for the development of diabetic cardiomyopathy (DCM). Following moderate diabetes, with the reduction in glucose utilization, the heart increases its LPL activity at the vascular lumen due to an increase in Hpa action. Although this adaptation might be beneficial to compensate for the underutilization of glucose by the heart, it is toxic over the long term, as harmful lipid metabolite accumulation, along with augmented FA oxidation and thus oxidative stress, leads to cell death. This coincides with the loss of a cardioprotective growth factor—namely, vascular endothelial growth factor B (VEGFB). This review discusses interconnections between Hpa, LPL, and VEGFB and their potential implications in DCM. Given that mechanism-based therapeutic care for DCM is unavailable, understanding the pathology of this cardiomyopathy, along with the contribution of LPL, will help us advance its clinical management.

1. Background

In individuals who live with diabetes (both Type 1 diabetes (T1D) and Type 2 diabetes (T2D)), heart disease is a major reason for mortality [1,2]. A significant cause of this cardiovascular disease is suggested to be atherosclerosis; however, heart failure can also result from a defect in the cardiac muscle, termed diabetic cardiomyopathy (DCM) [3–7]. DCM is defined as the occurrence of myocardial dysfunction (abnormal cardiac structure, left ventricle diastolic dysfunction, and reduced left ventricle ejection fraction) in the absence of coronary artery abnormalities, valvular defects, hypertension, and hyperlipidemia [7]. The mechanism behind the development of DCM is complex, but one major instigator is early changes in cardiac metabolism [4,8]. In diabetes, the heart reduces its utilization of glucose but increases its consumption of fatty acids (FAs) to generate adenosine triphosphate (ATP) [8,9]. Although FAs are provided to the heart from numerous sources, the majority of this substrate originates from plasma lipoprotein–triglyceride (TG) hydrolysis [10]. This is facilitated by lipoprotein lipase (LPL), an enzyme localized in the coronary lumen. In rats with mild diabetes characterized by low plasma insulin and high glucose, when the plasma concentrations of circulating FAs or TGs are within a normal range, coronary lumen LPL activity is augmented [11–13]. Although this early adaptation might be beneficial to compensate for the underutilization of glucose by the heart [14], it is toxic over the long term, as FA oxidation causes oxidative stress—a leading stimulus for initiating cell death [15,16]. An additional issue is that FA utilization requires proportionally more oxygen (O_2) than glucose utilization does to produce an equivalent amount of ATP [17]. This may be problematic, as the heart exhibits small-vessel disease (microangiopathy) following diabetes onset. Under these conditions, the increased provision

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of FAs cannot be matched with the O_2 supply, leading to incomplete FA oxidation, lipid metabolite accumulation, TG accumulation, ceramide synthesis, and cell death [18–22]. Intriguingly, in mouse models where LPL is exclusively overexpressed in the heart, the animals under study also exhibit cardiomyopathy, similar to that seen with DCM [23,24]. Following the onset of severe diabetes, with the extreme loss of insulin, when plasma FAs are augmented in addition to glucose, coronary LPL activity is reduced as a means of preventing lipid overload [11,25]. This process could be equally detrimental, because—with cardiac-specific LPL deletion at least—there is reduced ejection fraction [26,27]. As chronic treatment of T1D is associated with numerous incidences of inadequate management of hyperglycemia, this review will discuss potential mechanisms that lead to changes in cardiac LPL. Given that mechanism-based therapeutic care for DCM is unavailable [28], understanding the pathology of this cardiomyopathy and the contribution of LPL will help us advance the clinical management of DCM.

2. Diabetic cardiomyopathy

Evidence of heart dysfunction (i.e., DCM) has been reported in individuals with T1D and T2D, even though these patients do not exhibit atherosclerosis [7,29–31]. Similarly, DCM has been reported in animals with induced diabetes [6,32]. A number of etiologic mechanisms have been proposed for DCM, including the buildup of dense connective tissue, altered responses to different hormones (e.g., catecholamine), deficiencies in mitochondrial function (i.e., defects in mitochondrial structure and respiratory capacity), endoplasmic reticulum stress, activation of the renin–angiotensin–aldosterone system (RAAS), microangiopathy, and alterations in proteins that regulate intracellular calcium [7,31,33–36]. Our lab and others have also implicated alterations in cardiac metabolism as a key contributor toward the etiology of DCM [3,37–40].

3. Cardiac metabolism

Due to its constant pumping and contraction, the heart requires a substantial amount of energy. In this regard, the cardiac muscle can attain ATP from multiple substrates, including glucose, FAs, ketones, pyruvate, and amino acids [41]. Of these, FAs appear to be the major substrate that the heart prefers for energy generation [42]. Even though the heart prefers FAs, this organ is incapable of making this substrate by lipogenesis and depends on acquiring it from multiple processes: ① the adipose tissue lipolysis of stored TG, with the eventual transport of released FAs to the heart; ② stored lipid TG lipolysis; and ③ circulating lipoprotein–TG hydrolysis by vascular lumen LPL [3,9]. Of these, LPL-derived FAs are suggested to be the key source of FAs for cardiac energy generation [42].

4. Lipoprotein lipase

4.1. Overview

Of the various tissues that express LPL, including adipose tissue, lung tissue, and skeletal muscle, the heart is the organ with the highest expression of this enzyme. In addition, the majority of FAs in the plasma are present within circulating lipoproteins. These observations suggest that the action of LPL in breaking down lipoproteins allows for a significant provision of FAs for ATP generation in the heart [3]. It should be noted that, in adipose tissue, LPL controls FA entry for storage as TGs; excellent reviews are available on this topic for interested readers [43,44]. Lipoprotein–TG lipolysis occurs at the coronary luminal surface of endothelial cells (ECs). Notwithstanding this location, ECs are unable to synthesize LPL. Rather, it is produced in cardiomyocytes and subsequently secreted to its cell-surface heparan sulphate proteoglycan (HSPG) binding sites [45,46]. For LPL to move to the EC lumen, detachment of LPL from the cardiomyocyte surface HSPG is a prerequisite and is facilitated by heparanase (Hpa). From here, LPL attaches to glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) at the EC basolateral side [47] and is transferred to the apical lumen, ready for its function to generate FAs (Fig. 1) [47,48].

4.2. GPIHBP1

Multiple hypotheses have been suggested to elucidate how LPL moves from the cardiomyocytes, across the EC, to the vascular lumen. These include the transfer of the enzyme by endothelial-HSPG [49,50] and by the very-low-density lipoprotein (VLDL) receptor [51]. In a more recently proposed pathway, GPIHBP1 mediates the shuttling of LPL across ECs to the apical side [47,52]. GPIHBP1 is expressed exclusively in ECs. It chaperones LPL by means of its acidic domain electrostatically interacting with the enzyme, as *GPIHBP1* mutations in the acidic domain fail to bind LPL [53]. Recent studies have reported that LPL is active as a monomer and associates with GPIHBP1 as such in a 1:1 ratio [54–56]. In addition to its role in LPL shuttling, GPIHBPI at the apical side strongly binds lipoproteins (chylomicrons or VLDL)—an action that is mediated by LPL [53]. In this way, it serves as a stage for lipoprotein breakdown at the coronary lumen [57]. A third function of GPIHBP1 is that, by binding LPL, it is capable of stabilizing the enzyme, thereby preventing its inhibition by angiopoietin-like 3/4 (ANGPTL3/4) [58]. Given these important functions, mice deficient in GPIHBP1 exhibit profound increases in plasma TGs. Moreover, humans with GPIHBP1 deficiencies have developed hypertriglyceridemia (Fig. 1) [59,60]. At present, we are unaware of specific changes in cardiac ANGPTL3/4 and GPIHBP1 in diabetic human subjects. However, in animal studies, following a single injection of a moderate dose (55 $mg \cdot kg^{-1}$; D55) of streptozotocin (STZ), there is an induction of hypoinsulinemia and hyperglycemia. Increasing the dose to 100 $mg \cdot kg^{-1}$ also creates an environment of hyperlipidemia [61]. In the former situation, coronary LPL activity is augmented; in the latter setting and with the presence of higher levels of circulating FAs, LPL activity is turned off [11] (Fig. 1). With D55 hearts, in the absence of any change in protein synthesis, the increase in LPL activity—which principally occurs at the vascular lumen—could be largely explained by alterations in LPL secretory and signaling pathways that increased the transfer of myocyte enzyme to ECs [62]. To determine whether the increased vascular content of LPL following D55 diabetes is associated with GPIHBP1, the protein and messenger RNA (mRNA) expression of *GPIHBP1* were examined and were determined to be augmented [63]. In relation to ANGPTL4, we have reported that, in moderate and severe diabetes, cardiac gene expression increased ten- and twenty-fold, respectively [14]. Interestingly, although *ANGPTL4* increased ten-fold in moderate diabetic animals, this was not associated with a decrease in LPL activity; in fact, LPL activity increased three-fold [14]. We suggested that, even though *ANGPTL4* increased ten-fold, STZ-induced diabetes increased *GPIHBP1* gene and protein expression [63]. Hence, when LPL transfers onto ECs and complexes with GPIHBP1, this structure appears to protect LPL from inactivation by ANGPTL4. With severe diabetes, the unprecedented twenty-fold increase in *ANGPTL4* is likely sufficient to inhibit LPL activity.

4.3. Regulation

Different physiological states can sensitize LPL activity, and this can vary among tissues. For example, under caloric deprivation,

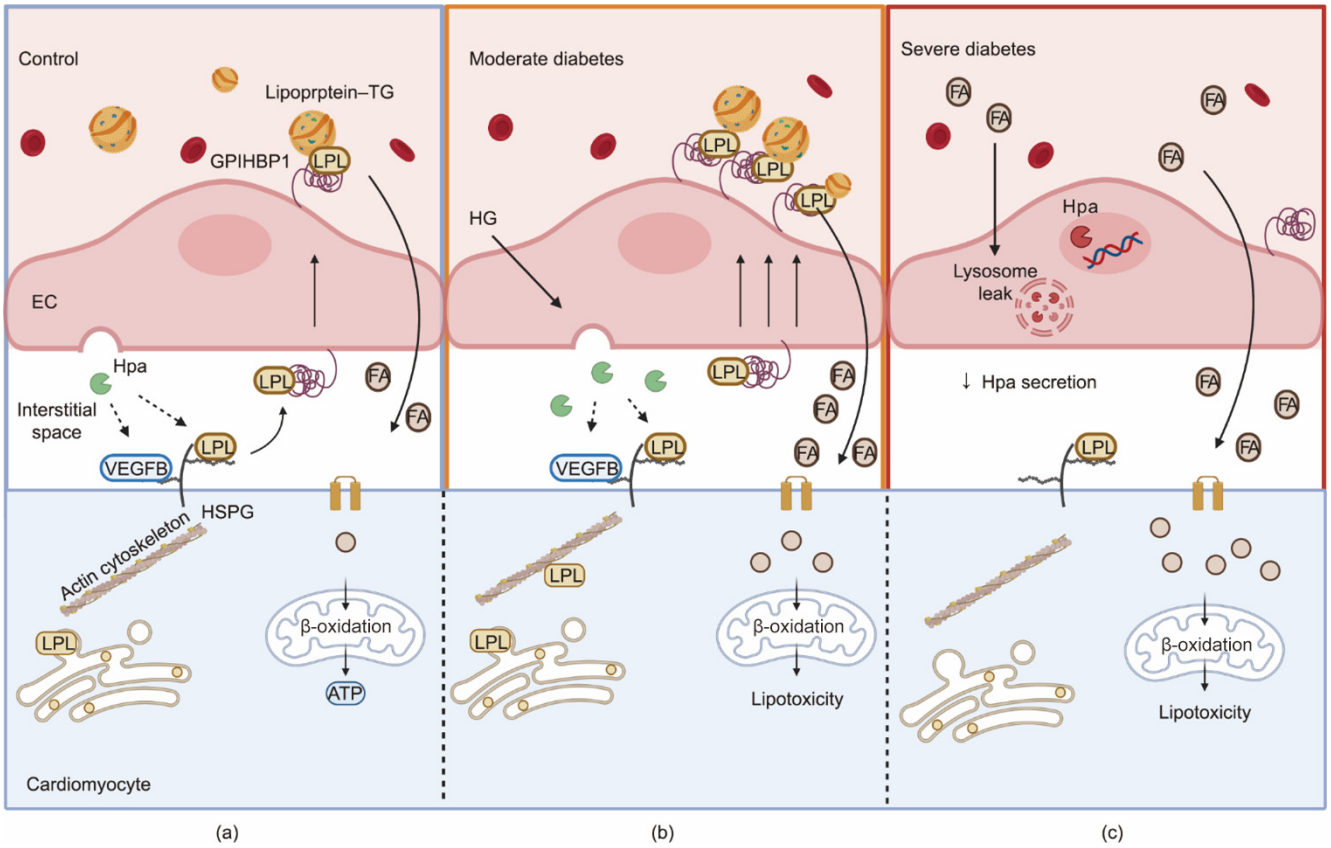

Fig. 1. LPL trafficking in the heart. (a) LPL is synthesized in cardiomyocytes and uses the actin cytoskeleton to move to cell-surface HSPGs. HSPGs house multiple proteins, including growth factors such as vascular endothelial growth factor B (VEGFB). For the onward transfer of LPL, HSPG side chains require cleavage, a function facilitated by the Hpa released from ECs. GPIHBP1 at the basolateral side of an EC captures LPL and transfers it to the apical side of the coronary lumen. At this location, LPL promotes lipoprotein–TG lipolysis to release FAs. These FAs are in turn taken up by the cardiomyocyte for ATP generation within the mitochondria. (b) In response to moderate diabetes, hyperglycemia causes secretion of endothelial Hpa, which cleaves HSPG-bound LPL from cardiomyocytes and promotes the replenishment of this HSPG-released LPL for onward translocation to the vascular lumen. At this location, LPL promotes lipoprotein–TG breakdown and the release of FAs for entry to the cardiomyocytes for ATP generation. In addition to releasing LPL, Hpa causes the release of VEGFB, whose action promotes protection against cell death and angiogenesis for oxygen supply. This VEGFB-mediated protection is lost following diabetes onset. HG: high glucose. (c) With severe diabetes and the presence of hyperglycemia and hyperlipidemia, Hpa is directed into the endothelial nucleus, preventing its secretion toward the basolateral side where the cardiomyocytes are located. Consequently, LPL fails to move toward the vascular lumen; in this case, the majority of cardiac energy is then provided by adipose tissue FAs.

adipose tissue LPL activity is reduced. This prevents the needless storage of substrates, making them available for energy generation in other tissues such as the heart [64]. Consequently, FAs generated from lipoprotein–TG are used for fulfilling the ATP requirements of the heart. In this way, LPL acts as a "door" to modulate tissue-specific demands for FAs.

4.4. Function in cardiomyopathy

When present in excess, FAs are diverted from adipocytes to alternative organs, including the heart. One unfortunate consequence of this effect is that FAs can trigger a decrease in cardiac efficiency, low glucose oxidation rates, structural impairments, and cellular demise [9,18–20,65]. In this regard, when LPL is specifically overexpressed in the heart, more FAs are provided, which has been reported to cause severe muscle defects including cardiomyocyte apoptosis, as well as reduced function in the absence of vascular changes—a situation comparable to DCM [23,24]. Conversely, the experimental removal of LPL only from the heart is also known to cause cardiomyopathy [26,27]. Even though these hearts were still able to use albumin-bound free FAs (FFA) and increased their glucose utilization, these actions could not substitute for the function of LPL; thus, heart function was also reduced [27]. Taken together, these results suggest that cardiac failure can be induced simply by altering cardiac LPL.

4.5. Cardiac LPL in patients with diabetes

In humans, the highly negatively charged glycosaminoglycan, heparin, is employed to displace HSPG-bound LPL into the plasma. This enables the subsequent quantification of its plasma activity [45,66]. This method is not ideal as, in addition to releasing LPL from the heart, it releases LPL from several other tissues such as the skeletal muscle and adipose tissue. Therefore, this procedure cannot be used to determine the heart-specific impact of diabetes on cardiac LPL. When evaluating how diabetes impacts LPL in different tissues, such as adipose tissue and skeletal muscle, it was observed that these organs demonstrated considerably lower LPL [67]. Unfortunately, there is limited amount of data on the distribution of LPL in the heart. Even if it were possible, measuring total cardiac tissue LPL would be flawed, as such measurement would be unable to identify the relevant pool of LPL at the coronary lumen. Due to these limitations in human analyses, the vast amount of data related to cardiac LPL in diabetes has been obtained from animal studies.

4.6. Cardiac LPL in animal models of diabetes

In models of drug-induced insulin resistance [68,69] or STZ-induced diabetes with moderate hypoinsulinemia and hyperglycemia in rats [11,13,61,70], LPL activity was increased at the coronary

lumen—an effect that was reversible with exogenous insulin treatment [13]. In this STZ model, the increase in LPL activity did not occur due to an increase in HSPG binding sites. In fact, we determined that, in a normal heart, EC binding sites at the vascular lumen are only fractionally occupied by LPL. In diabetes, the vacant sites are immediately occupied by LPL [13,62,71], which does not involve gene and protein expression changes [13,72]. Rather, secretory and signaling pathways are altered, which encourages the vectorial movement of cardiomyocyte LPL to the EC apical side [39,61]. This includes the activation of adenosine monophosphate-activated protein kinase (AMPK) [73,74], p38 mitogen-activated protein kinase (p38MAPK), and protein kinase D (PKD) [12,25,75], which results in the secretion of LPL onto cardiomyocyte-surface HSPG, involving the formation of vesicles containing LPL and reorganization of the actin cytoskeleton [75,76]. For LPL to move forward from this location, it requires detachment from the cardiomyocyte cell surface—an effect that is mediated by the cleavage of HSPG through the action of Hpa [77,78] (Fig. 1). In this regard, we reported that, in response to high glucose, ECs release Hpa [77,79], which mostly occurs from the basolateral side [80]. This in turn promotes a release of LPL from cardiomyocytes [81]. Intriguingly, we also demonstrated that, in addition to releasing LPL, Hpa can liberate cardiomyocyte cell surface growth factors including vascular endothelial growth factor A (VEGFA) [82,83] and VEGFB [84]. By modulating oxygen delivery and preventing cell death, both of these growth factors can defend against the excessive use of FAs. It should be noted that, in models with severe diabetes [11,61], there is an increase in plasma FAs due to unregulated adipose tissue lipolysis. In this regard, diabetic animals exhibit close to a two- to three-fold increase in various types of saturated (palmitic (16:0), stearic (18:0)), monounsaturated (oleic (18:1)), and polyunsaturated (linoleic (18:2), arachidonic (20:4)). FAs that make up about 80% of the total plasma pool [14]. We suggested that LPL-mediated FA delivery would be redundant in these circumstances and is reduced.

5. Heparanase

5.1. Overview

In tissues, HSPGs are located at multiple sites, especially the extracellular matrix and nucleus [85]. They consist of a central protein to which a number of heparan sulfate (HS) side chains are bound. These molecules thus offer structural integrity to the cell membrane, in addition to anchoring several molecules due to the highly negatively charged groups in HS [86]. The negatively charged HS side chains are used to attach several positively charged proteins, including C–X–C motif chemokine ligand 2 (CXCL2), thrombin, LPL, VEGFA, and VEGFB. Due to this ionic attachment, these proteins can be immediately released when required. The endo-β-glucuronidase Hpa is unique in that it is the only known mammalian enzyme to cleave HS, thereby promoting the abovementioned release of proteins (Fig. 1) [87].

5.2. Secretion and response to high glucose

Hpa is an enzyme that is able to cut HS side chains, resulting in the liberation of bound proteins [88]. It is manufactured in the EC endoplasmic reticulum (ER) as a 68 kDa protein that is then processed into a 65 kDa inactive latent Hpa (Hpa^L). Hpa^L is then secreted and rapidly endocytosed back into the EC [89,90] via HSPG and receptors such as the mannose-6-phosphate receptor and LDL-receptor related protein 1 [91]. Hpa^L is processed in early endosomes and lysosomes into active Hpa (Hpa^A) by cathepsin L under acidic conditions. Cathepsin L removes a 6 kDa linker, resulting in 8 and 50 kDa subunits that noncovalently heterodimerize, resulting in Hpa^A [92]. Hpa^A, which has been shown to be 100 times more active than Hpa^L, is stored in lysosomes until it is stimulated for release [93,94]. Our lab has shown that high glucose is a robust stimulus for EC Hpa^A release into the medium. This secretion is through high glucose-stimulated ATP release, resulting in purinergic receptor (P2Y) stimulation, actin reorganization, and Hpa^A vesicle release [77]. Conversely, unlike high glucose conditions, high FA conditions prevent Hpa secretion by redirecting Hpa into the nucleus [95].

5.3. Functions

Physiologically, Hpa^A is involved in embryonic implantation, wound repair, and hair follicle maturation [96]. In relation to cardiac metabolism, we first reported the distinct function of high glucose in releasing EC Hpa, with the subsequent liberation of myocyte LPL. This allows for the forward movement of LPL to the vascular lumen, where it facilitates lipoprotein–TG breakdown, providing the diabetic heart with FAs as an energy source [78]. In addition to Hpa^A, high glucose stimulates the secretion of Hpa^L [82]. We determined that Hpa^L is able to produce intracellular signals in cardiomyocytes, permitting LPL reloading. This allows for the refilling of the HSPG binding sites previously occupied by LPL. It should be noted that, although both forms of Hpa promote several cell signaling pathways as well, including protein kinase B (Akt), extracellular signal-regulated kinase (Erk), proto-oncogene tyrosine-protein kinase (Src), signal transducer and activator of transcription proteins (STAT), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF) [97], Hpa^L is more effective as a VEGF-releasing stimulus (Fig. 1) [82,84].

6. Vascular endothelial growth factors

6.1. Overview

Six growth factors are included in the VEGF group of proteins: VEGFA, VEGFB, VEGFC, VEGFD, VEGFE, and PGF [98]. The most extensively examined of these is VEGFA, which is considered to be especially important for controlling angiogenesis [99]. Interestingly, VEGFB does not directly initiate angiogenesis [100–102]. This paradigm has been revisited, and more current research has suggested that VEGFB plays a role in angiogenesis by indirectly sensitizing tissues to VEGFA [103,104]. Other important roles of VEGFB include its ability to prevent cell death [102], which could be especially relevant in diseases such as diabetes [103], and will thus be discussed in detail.

6.2. VEGFB

In tissues that demonstrate higher oxidative capacity, including the heart and skeletal muscle, VEGFB shows its highest expression [105]. The function of VEGFB occurs through its binding to VEGF receptor-1 (VEGFR1). As the homology of VEGFB to VEGFA is 47% identical [105], much effort has been spent on examining its contribution to angiogenesis—experiments that proved inconclusive. A more recent suggestion is that VEGFB does in fact lead to new vessel formation by supporting VEGFA in its angiogenic function. VEGFA is known to bind both VEGFR1 and VEGFR2; however, the former receptor has a ten-times greater binding capacity for VEGFA [106], albeit with few downstream effects. This suggests that the binding of VEGFA to VEGFR1 restricts its angiogenic action [107]. Accordingly, *VEGFR1* knockout [108] or *VEGFA* overexpression [109] are embryonically lethal as, under these conditions, only

VEGFR2 is occupied by VEGFA, leading to profound and unregulated angiogenesis. In another example, mice transduced with adeno-associated virus (AAV)–VEGFB demonstrated both vessel enlargement and an increase in blood vessel number in adipose tissue [104]. The researchers suggested that this effect of VEGFB was a result of its occupation of VEGFR1, which reduced VEGFA's ability to interact with VEGFR1 and caused VEGFA to exclusively bind to VEGFR2, with obvious effects on the vasculature. Under these particular conditions, the vasculature of AAV–VEGFB mice was normal and divergent from what was seen with AAV–VEGFA mice, which showed abnormal vasculature—suggesting a normal effect of VEGFB on blood vessels without any harmful outcomes.

6.3. Impact on whole body and cardiac metabolism

Mice fed a high-fat diet and injected with AAV–VEGFB exhibited improvement in insulin action [104]. This could be explained in two ways: either through the direct effect of VEGFB on organs such as the skeletal muscle, adipose tissue, and liver, or indirectly, by VEGFB augmenting vascular development, resulting in a greater distribution of insulin to the aforementioned organs. In relation to cardiac metabolism, rat hearts that specifically overproduced VEGFB exhibited increased intracellular transport of glucose and higher glycolytic capacity, indicating greater carbohydrate utilization for energy production [103]. Conversely, these rats exhibited decreased expression of genes related to FA transport and oxidation [103], indicating that VEGFB transforms the heart from predominately using FAs to a reliance on glucose.

6.4. Influence on cell survival

Both human and experimental animal studies have demonstrated the beneficial impact of VEGFB on prolonging cellular longevity. Hence, in patients with heart failure undergoing transplant surgery, unhealthy hearts display decreased *VEGFB* gene expression [103]. In cell culture experiments in which ECs or smooth muscle cells were obtained from animals lacking VEGFB, inducing oxidative stress by means of H_2O_2 was found to accelerate regulated cell death (apoptosis)—an effect that was minimized under treatment with exogenous VEGFB [110,111]. Finally, when VEGFB is provided through either purified protein or viral transduction to increase endogenous production, the heart is protected against the damage induced by oxidative stress [84], aortic banding [112], arrythmias [113], doxorubicin [114], and ischemia [115].

6.5. VEGFB in diabetes

The function of VEGFB has been well established, especially as it relates to cardiac substrate utilization, blood vessel formation, and cell death prevention. Similar to its release of LPL, Hpa has been reported to release VEGFB, especially following acute diabetes [82]. This effect, when taken together with the function of VEGFB to protect against cell death and to increase the coronary vasculature, offers a mechanism to defend against lipotoxicity. With the loss of VEGFB following severe or chronic diabetes [84], the VEGFB protective effects are lost and the provision of LPL-derived FAs is unchecked. Indeed, although diabetes exhibits altered metabolic inflexibility [116], microvascular rarefaction [36,117,118], and cardiomyocyte demise [30,119,120], all of these could be secondary to VEGFB loss. This data provides compelling primary evidence that a reduced level of VEGFB may promote the development of diabetic heart failure [84].

7. Concluding remarks

In this study, we reported that, in response to high glucose, the EC release of Hpa and its subsequent action on LPL liberation from cardiomyocytes enables substrate switching to the utilization of FAs. Counter-balancing this effect, Hpa can also release cardiomyocyte VEGFB, which can ① affect angiogenesis, ② promote glucose utilization, and ③ protect against cell death.

In a situation with augmented LPL activity and the loss of VEGFB, lipotoxicity and cell death are consequences that lead to DCM. Thus, understanding the network that connects vascular endothelial Hpa with cardiomyocyte LPL and VEGFB is important for determining how to maintain heart function, especially under disease conditions such as diabetes.

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Compliance with ethics guidelines

Chae Syng Lee, Yajie Zhai, and Brian Rodrigues declare that they have no conflict of interest or financial conflicts to disclose.

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